INVASIVE INTERACTIONS OF *Monomorium minimum* (HYMENOPTERA: FORMICIDAE) AND *Solenopsis invicta* (HYMENOPTERA: FORMICIDAE) INFECTED WITH *Thelohania solenopsae* (MICROSPORIDA: THELOHANIIDAE)

A Thesis

by

MOLLY ELIZABETH KECK

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2005

Major Subject: Entomology

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Approved as to style and content by:

Roger E. Gold (Chair of Committee)	Norman O. Dronen (Member)
S. Bradleigh Vinson (Member)	Kevin Heinz (Head of Department)

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ABSTRACT

Invasive Interactions of *Monomorium minimum* (Hymenoptera: Formicidae) and *Solenopsis invicta* (Hymenoptera: Formicidae) Infected with *Thelohania solenopsae* (Microsporida: Thelohaniidae). (May 2005)

Molly Elizabeth Keck, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Roger E. Gold

Thelohania solenopsae Knell, Alan, and Hazard is an internal microsporidian that parasitizes the red imported fire ant, *Solenopsis invicta* Buren. This experiment studied the invasive interactions between the native United States ant, *Monomorium minimum* (Buckley), and *S. invicta* colonies infected with *T. solenopsae* and *S. invicta* colonies free of parasites. This study utilized *S. invicta* colonies of 100, 300, 600, 800, and 1000 workers to determine the ability of 1000 *M. minimum* workers to invade each *S. invicta* colony size. There was a significant difference in the time for *M. minimum* to invade *S. invicta* when comparing *S. invicta* colonies of 1000 workers infected with *T. solenopsae* to *S. invicta* colonies that were uninfected. It was also determined that there was a significant difference in the time for *M. minimum* to invade smaller uninfected *S. invicta* colonies as opposed to larger uninfected *S. invicta* colonies. There was no significant difference in the ability of *M. minimum* to invade smaller *S. invicta* colonies infected with *T. solenopsae* as opposed to larger infected *S. invicta* colonies. It was therefore concluded that *S. invicta* colonies infected with *T. solenopsae* were not able to

defend their colony or prevent competing ants from invading as well as uninfected *S. invicta* colonies. This study also demonstrated that *M. minimum* is a significantly more invasive species when compared to *S. invicta,* invading *S. invicta* territories in every situation and doing so in a significantly shorter period of time than *S. invicta* colonies invaded *M. minimum* colonies.

DEDICATION

There are several people to whom I would like to dedicate this thesis. First and foremost, to my parents, Russell and Martha Bush, who have loved, supported, and cheered me on in everything I have done throughout my entire life. At least one of you has been there to witness all the important moments in my life, from games and track meets to awards ceremonies and graduations. You have been a constant pillar of strength and I have always felt supported. To my father who has taught me the importance of hard work, dedication and most importantly, education: I can only hope to one day be as successful and knowledgeable in my field as you are in yours. I truly believe that using you as a role model has gotten me to where I am today. To my mother whose love and enthusiasm for everything I have ever attempted in my life has made it all worthwhile. You have had to spread your love, attention, and time evenly over four needy children, and you have done so gracefully.

Finally, I would like to also dedicate this thesis to my husband, Casey. Thank you for always listening to me and being the voice of reason in my life. You were a wonderful help, risking your health by collecting fire ants with me on weekends and joining me at the lab on late nights to help me count ants. You kept me sane when the experiments didn't seem to work and brainstormed with me to figure out what went wrong. You were always concerned about my results and progress and it made this whole process much more meaningful to have someone to share it with.

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Thank you to everyone at the Center for Urban and Structural Entomology. The past two years have been a pleasure because of you: Laura Nelson for all your administrative help; Dr. Harry Howell, Jr. for all your statistical advice and assistance; and Dr. Mark Wright for advice on everything from chemicals to computers to life. The friendship and advice I received from my fellow graduate students: Bryan Heintschel, Anne Narayanan, Barry Furman, Kim Engler, Grady Glen, and Jason Meyers was vital to the completion of this degree. I would like to extend a special thanks to Bryan Heintschel and Jill Millican for thinking of me during their vacation time by searching for and collecting ants in San Angelo, TX and transporting them back to the lab. You

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INTRODUCTION

Solenopsis invicta Buren, the red imported fire ant (RIFA) (Hymenoptera: Formicidae), was introduced into the United States at Mobile Alabama between 1933 and 1945 from South America (Lofgren et al 1975). RIFA's introduction was accidental, and it is believed that the mode of transport was on in the ballast of a ship carrying goods to the United States (U.S.). However it will probably never be determined exactly how RIFA became established in the U.S. because RIFA has not been directly associated with any cargo that could likely house a colony or mated queen, and there have been no records of anything having been received during the time frame in which RIFA is believed to have entered the U.S. (Buren et al. 1974, Lofgren et al. 1975).

Since its introduction, RIFA has spread fairly rapidly throughout the southeastern U.S., and is now considered the dominant ant species in Texas, infesting approximately the eastern one third of the State (Porter et al. 1991). RIFA infests 14 southern states in the U.S., ranging from Maryland to California, covering 128 million hectares in the nine most heavily infested states (Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina and Texas).

This thesis follows the format of the Journal of Economic Entomology.

RIFA has spread efficiently and quickly due to their nuptial mating flights, wherein females can travel miles from their original nest, encouraging movement from county to county (Lofgren et al. 1975). Humans also facilitate the spread of RIFA to other parts of the country by transporting items in which RIFA have formed colonies (Lofgren et al. 1975). This allows them to travel much quicker than by their own dispersal. RIFA may also be distributed to other areas during floods and irrigation of agricultural systems by balling up into a mass of ants and floating to different areas (Bhatkar and Gold 1991).

RIFA are eusocial insects, meaning that their colonies exhibit such characteristics as overlapping generations, communal care for the young, and a division of labor (or a caste system) in which one or more non-reproductive castes are present (Borrer et al. 1992). Colonies are made up of brood (eggs, larvae, and pupae), workers, queens, and male and female reproductives, which are commonly called alates because they possess wings. Workers are female and have the ability to sting. Workers are also polyethic, in which they have different duties within the colony based on their age. The youngest workers, commonly called nurses, feed and clean the members of the colony. Slightly older workers are called reserves and are responsible for colony maintenance, sanitation and defense. The oldest workers of the colony are foragers and leave the colony in search of food (Vinson 1997).

The female reproductive mates in the air during a nuptial flight and will return to the ground to found a new colony by shedding her wings, excavating her burrow, and plugging the entrance. The queen's first eggs will be laid 24-48 hours after excavation

of the nest. Approximately 30 days after the first eggs are laid the first workers will appear (Lofgren et al. 1975). Colonies are considered mature after three years, and may contain up to 400,000 workers (Logren et al. 1975, Vinson 1997). Queens can lay up to 800 eggs per day and reportedly can survive seven or more years, only mating once during her lifetime. Workers live for approximately five weeks, although larger workers can live longer (Drees et al. 1996).

Compounding to the extremely high reproductive potential of RIFA is the fact that colonies can exist in two forms: monogyne, in which there is a single queen, and polygyne, in which there are multiple queens. In Texas, polygyne is the dominant colony form (Porter et al. 1991). Polygyne colonies are considered more detrimental than monogyne colonies because there is little to no aggression between workers of the different colonies and this combination allows for a greater density of mounds per unit area (Porter et al. 1991, Vinson 1997, Williams et al. 1998).

RIFA's pest status includes medical, urban, agricultural, and ecological implications, making control of this species an important issue. Medically RIFA are important because of the sting they inflict during defense. RIFA are extremely aggressive ants that are able to sting more than once, releasing venom from a venom sac located in their abdomen each time the stinger is inserted into the skin. The most common reaction to RIFA stings is the formation of pruristic pustules, which are subject to secondary infection if not cared for properly (Deslippe and Guo 2000). Sensitive individuals may experience localized swelling, anaphylactic shock, and in very rare cases, death may occur (Rhoades 1997).

As an urban pest, RIFA mounds are unappealing aesthetically and cause medical concerns. In addition, RIFA are attracted to electric fields and will form nests in electrical equipment, causing circuits to ground and short, and preventing electrical connections from being made (Vinson and MacKay 1990). It was estimated that $580 million was spent in the major cities and metroplexes in Texas for damages and expenditures due to RIFA from 1998 to 1999 (Lard et al. 2001).

RIFA stings also have negative adverse affects on wildlife and livestock. Ground nesting birds, deer, and reptiles are particularly vulnerable to RIFA attack (Drees and Knutson 2002). Barr and Drees (1994) estimated that in 1993 pet and livestock owners spent $750,000 annually to treat RIFA related injuries for over 7,200 animals. In addition, the authors estimated that $4.5 million was lost yearly in pet and livestock deaths believed to be caused by RIFA. Small animals and pets are the most frequently treated animals for RIFA related health problems, followed by cattle and wildlife (Barr and Dress 1994).

In addition to agricultural damage related to livestock, RIFA can also cause damage to crops. Crops that have been reported in scientific literature to be damaged by RIFA include okra, corn, soybean, potatoes, beans, cabbage, sorghum, and long leaf pine seedlings (Lofgren et al. 1975, Apperson and Adams 1983, Thompson 1990). Hay pastures severely infested with RIFA often cannot be cut, resulting in economic losses (Lofgren et al. 1975).

Ecologically, RIFA disturb the abundance of other organisms in the environment because their aggressive and invasive nature allows them to displace other organisms, as

well as eliminate the food used by certain wildlife (Drees and Knutson 2002). Porter and Savignano (1990) found that the invasion of polygyne colonies in Texas produced major changes the in abundance and diversity of other ants and surface-active arthropods. RIFA was found to be especially damaging to the communities of native ants.

Because RIFA is not a species native to the U.S., it was introduced with very few natural enemies, necessitating control measures by man. Currently, the most common source of control for RIFA is through the use of pesticides (Pereira and Stimac 1997)). Although this has historically been shown to be the most successful and fastest acting control for RIFA, the development of an appropriate biological control program remains an important entomological objective (Drees and Gold 2003, Lofgren et al. 1975). Alternate sources of control are needed in situations in which other native and beneficial insects could potentially be adversely affected by pesticides, in large areas where broad scale pesticide application is unrealistic, and in areas where there are pesticide restrictions.

Biological control can be more beneficial and appealing than chemical control for a variety of reasons. Biological control is environmentally safe, and if the program is developed and implemented correctly, adverse affects to other organisms can be avoided. Biological control can also be more economical than the use of pesticides, because biological control agents are self-perpetuating, keeping their populations present without additional help from humans. This reduces the cost of chemical control either

by eliminating the need for pesticides completely or reducing the amount and frequency of supplemental pesticide application.

Over 30 natural enemies have been discovered in RIFA's native South America, and several of them have been used as biological control agents in the U.S. (Porter 1998). Two species of fungi, *Beuvaria bassinia* (Balssano) and *Metarhizia anisoplisae* (Metschnikoff), have proved to be effective in controlling RIFA, causing 80% and 40% mortality, respectively, in RIFA colonies five to ten days after exposure (Lofgren et al. 1975). A parasitic phorid fly, *Pseudacteon* spp. has been shown to help in the control of RIFA by inhibiting foraging and producing larvae that decapitate RIFA workers and use the empty head capsule as a pupal case (Porter 1998). Two nematode species, *Steinernema carpocapsae* Weiser and *Heterorhabditis* spp. parasitize RIFA colonies, although colony relocation is a common result (Drees et al. 1992). Research by J.L. Cook (1996) found the strepsipteran parasitoid, *Caenocholax fenyesi* Pierce, is effective in controlling RIFA colonies by not only distressing the parasitized ants, but also weakening the entire colony by disrupting the social structure.

Microsporidia are generally considered to be the most important protozoan pathogens of insects and are considered to be the most promising protozoa group for microbial biological control (Undeen and Vavra 1997). Microsporidia are intracellular, obligatory parasites of protists, vertebrates, and invertbrates, including insects (Bigliardi and Sacchi 2001). Of the entomopathogenic microsporidia, *Thelohania solenopsae* Knell, Allen, & Hazard (Thelohaniidae) has been found to infect the genus *Solenopsis* (Briano et al. 2002). Certain characteristics are unique to microsporidia; they only exist

outside the host body as a spore, invasion of the host is accomplished through the use of an eversible polar tube, they have atypical eukaryotic ribosomes, and they are devoid of mitochondria or a typical Golgi apparatus (Bigliardi and Sacchi 2001).

Williams et al. made the first record of *T. solenopsae* in the United States in 1998. *Thelohania solenopsae* is found in the fat body of RIFA and has been shown to significantly reduce the weights of workers, reproductives, and queens (Cook et al. 2003, Williams et al. 1999). *Thelohania solenopsae* has been found in the abdominal fat body of workers, males, and queens, as well as the ovaries of queens and RIFA eggs (Knell et al. 1977, Williams et al. 1999). When RIFA are heavily infected with *T. solenopsae*, the infected fat body will undergo hypertrophy. The parasite will cause the formation of cysts, which emerge from severed gasters, the rounded part of the abdomen.

Infections by *T. solenopsae* result in a reduction of the overall fecundity of the colony. The number of eggs laid is reduced, lowering the brood volume, and decreasing worker populations (Williams et al. 1999 and Oi and Williams 2002). Infected queens also experience a decrease in queen weight (Williams et al 1999). In infected female reproductives the lipid stores are significantly fewer than healthy female reproductives (Overton 2003). Overton (2003) also suggested that *T. solenopsae* might be affecting the reproductive capability of RIFA by delaying the time when infected alates leave the colony. This author found that uninfected alates leave to perform their nuptial flights sooner than infected alates, therefore reducing the probability of infected female alates finding a mate.

The life cycle of *Thelohania solenopsae* begins with the infective stage, which is an environmentally resistant spore that is picked up by a RIFA worker and either ingested or transported back to the colony and fed to a sister worker, larvae, or queen. Once inside the host, an eversible coiled polar filament anchors itself to the host fat body cell and injects the spore contents into the cytoplasm of the cell (Undeen and Vavra 1997; Bigliardi and Sacchi 2001). When inside the fat body cell, *T. solenopsae* matures and reproduces and the offspring will exit the host as the environmentally resistant spore stage, either after the host has died or through the host's feces. Once *T. solenopsae* is back in the environment it is again ready to be picked up and ingested by another host (Undeen and Vavra 1997). In addition to horizontal transmission (transmission from individual to individual), *T. solenopsae* can also be transmitted vertically, from queen to the offspring.

Thelohania solenopsae is believed to be an excellent candidate as a biological control agent because along with the negative effects it inflicts upon RIFA colonies. It is found naturally in the soil and is not negatively affected by the high temperatures and dry summers of Texas (Cook 2002). *Thelohania solenopsae* is also able to persist in the environment in the absence of available hosts by forming resistant spores with a thick wall that protects it from harsh environmental conditions (Beglardi and Sacchi 2001, Dunn and Smith 2001). In the case of polygyne colonies, *T. solenopsae* can spread from colony to colony very easily as workers and brood travel between colonies (Williams et al. 1998; Naug and Camazine 2002).

Because RIFA colonies are stressed by the presence of parasites, it is likely that they are more vulnerable to other biological control agents or other competing organisms. One native ant that has been found nesting in close proximity to RIFA colonies is the little black ant, *Monomorium minimum* (Buckley). This ant is native to the U.S. and has been shown to successfully compete with RIFA for food and territory (Thompson 1990, Rao and Vinson 2004).

As an urban pest, *M. minimum* is known to nest in lawns, woodwork, building foundations, rotten wood, under objects, and occasionally enter a home or other structures, making it undesirable to humans (Smith 1965, Thompson 1990). The pest status of *Monomorium minimum* has lowered due in part to the invasion of RIFA, which has been successful in displacing many native ants (Porter and Savignano 1990). In comparison to RIFA, *M. minimum* have subterranean or cryptic nests that are not as noticeable as the mounds RIFA construct. *Monomorium minimum* possess a stinger, however the venom does not cause the formation of a pustule and no medical reactions have been recorded in humans (Thompson 1990). *Monomorium minimum* workers are much smaller than RIFA workers; 1.5 mm in length versus 2-6 mm in length (Thompson 1990).

The research objectives of this study were designed to examine the invasive interactions between *M. minimum* and RIFA. The first objective of this thesis was to determine whether *M. minimum* was deterred from invading larger RIFA colonies as opposed to smaller RIFA colonies. This objective tested the null hypothesis that there

was no significant difference in the ability of *M. minimum* to invade RIFA colonies of large worker numbers as compared to RIFA colonies of smaller worker numbers.

The second objective of these experiments examined if RIFA colonies infected with *T. solenopsae* are more susceptible to invasion by *M. minimum* than RIFA colonies that are not infected with *T. solenopsae* (these colonies will hereafter be referred to as healthy colonies). This study determined the time required for *M. minimum* to invade RIFA colonies after the initiation of invasion and tested the null hypothesis that there is no significant difference in the time for an invasive event to occur by *M. minimum* into RIFA colonies infected with *T. solenopsae* as compared to and healthy RIFA colonies. The third objective of this study was to compare the invasiveness of the two ant species, RIFA and *M. minimum*. This objective tested the null hypothesis that there is no significant difference in the invasiveness of RIFA colonies as compared to *M. minimum* colonies.

MATERIALS AND METHODS

RIFA colonies utilized in these experiments were collected from two sites in Brazos County, Texas (N30º37’21.06” W96º21’34.38” and N30.41º41’39.84” W96º20’33.66”), two sites in Burleson County, Texas (N30º31’21.78” W96º25’24.72” and N30º37’54.573” W96º40’59.776”), and one site in Guadalupe County, Texas (N30º41’19.14” W96º20’56.34”). RIFA were collected and maintained in the laboratory by means described by Banks et al. (1981) and contained in 40 x 27 x 8.5 cm plastic sweater boxes coated with Fluon® to prevent RIFA escape. *Monomorium minimum* colonies were collected from two sites: Brazos County, Texas (N30º36’20.52” W96º19’0.18”) and Tom Green County, Texas (N31º24’42” W100º28’59”). At both sites, *M. minimum* were collected from a rotten log in which the colony was nesting. The logs were broken into pieces and placed in 44.75 x 34.5 x 10 cm plastic bins, coated with a thin film of talcum powder to prevent *M. minimum* from escaping from the bin. The pieces of log were allowed to dry out in the laboratory, forcing *M. minimum* to travel from the log into 20 mL test tubes filled halfway with water and corked with cotton. After *M. minimum* colonies nested in the available water tubes, the tubes were placed into 31.75 x 17.15 x 9.5 cm plastic shoe boxes coated with Fluon®.

In order to initiate RIFA colonies for experiments, two dealate queens and brood from the collected colonies were placed in a petri dish filled with Castone® plaster that was moistened with deionized water; this served as the colony’s nest. The nest was housed in a 31.75 x 17.15 x 9.5 cm shoe box coated with Fluon®. The plaster was moistened weekly with water to ensure that the nest remained humid. Two 3 cm holes

were drilled into the Petri dish lids to allow workers and queens to enter and leave the nest at will. Colonies containing 100 workers were housed in 3.5 x 1 cm Petri dishes, colonies of 300 and 600 workers were housed in 5.3 x 1.2 cm Petri dishes, and colonies of 800 and 1000 workers were housed in 8.75 x 1.3 cm Petri dishes. The varying sizes of Petri dishes allowed action within the nest to be easily observed and furnished RIFA with a nest size correlating to the colony size.

One hundred workers were randomly counted out individually from one colony at each site and weighed to the nearest 0.0001 g. The weights for colony sizes of 300, 600, 800, and 1000 workers were then estimated based on the weight of the original 100 workers. These estimations were accurate, because both the original 100 workers and subsequent workers were randomly chosen without regard to worker size. The workers chosen for experiments were collected from all areas of the box and nest to ensure that nurses, reserves, and foragers would make up colonies.

The initiation of *M. minimum* colonies was performed by placing three dealate queens and brood in 31.75 x 17.15 x 9.5 cm plastic sweater boxes coated with Fluon®. One hundred workers were individually counted out from each site and weighed to the nearest 0.0001 g. The resulting mass was then multiplied by 10 in order to determine the weight of 1000 workers, and 1000 workers were utilized for each *M. minimum* colony used in the this study. Because *M. minimum* workers are monomorphic, these estimates are accurate. *Monomorium minimum* were provided 20 mL test tubes filled half way with water and corked with a cotton ball stopper to be utilized as nests. The nests were

covered with 8 x 6 cm dark construction paper folded in half, forming a tent over the test tube to provide the illusion of a dark, enclosed nest (Figure 1).

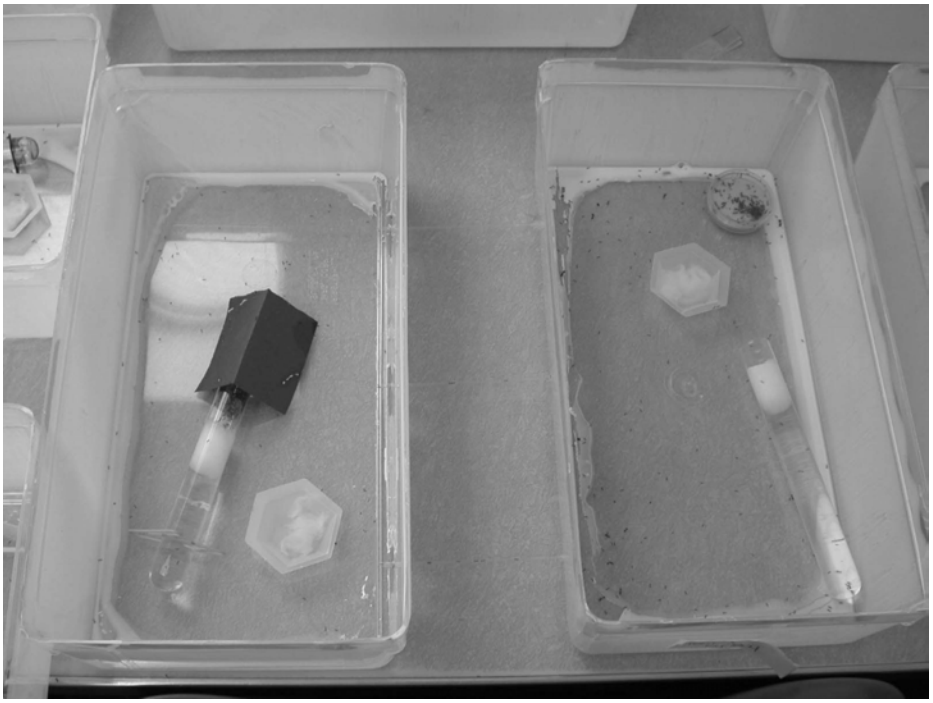

Figure 1. The experimental setup for *Monomorium minimum* and *Solenopsis invicta* colonies. *Monomorium minimum* colony boxes are shown on the left, and *Solenopsis invicta* colony boxes are shown on the right.

RIFA and *M. minimum* colonies were fed mealworms for protein and honey water as a carbohydrate source. Two medium mealworms were fed to both ant species three times a week (Monday, Wednesday, and Friday), and approximately one teaspoon of honey water was fed to both ant species once a day for the duration of the experiment. *Monomorium minimum* nests provided the colonies with water, and RIFA colonies were provided a test tube filled with water and stuffed with a cotton ball wick to serve as a

water source. When water tubes were devoid of water, new, unused tubes were placed in the shoeboxes.

All infected RIFA colonies were infected naturally in the field and no inoculation of the parasite was done. After collection of each colony, a trichrome stain was performed in order to determine the status of *T. solenopsae* infection (Weber et al. 1992). Approximately 10 to 40 workers were collected from each colony and placed into 1.5 mL sterile Eppendorf tubes. The Eppendorf tubes were then placed in a freezer at -10°C until all ants were incapacitated. Sixty to 100 µL of deionized water were added to the Eppendorf tubes and the ants were ground up using sterile tissue grinder pestles until the water turned cloudy. After allowing the Eppendorf tubes to settle, 30 µL of the subsequent homogenate were extracted and placed on a microscope slide. In order to prevent contamination, each colony sample was placed in separate Eppendorf tubes, ground up using separate sterile tissue grinder pestles and the homogenate was placed on separate microscope slides.

After slides were allowed to dry overnight, the staining process began. The staining process follows the same guidelines set forth by Weber et al. (1992). Slides were fixed in methanol for 10 minutes to dehydrate the dried homogenate so that the spores could absorb the water-soluble stain. After fixation, they were emerged in the chromotrope-based stain for 90 minutes. The stain consisted of 12.0 g of chromotrope 2R, 0.3 g of fast green, 1.4 g of phosphotungstic acid, and 6 mL of acetic acid, which was allowed to sit for 30 minutes before adding 200 mL of distilled water.

After the slides were stained, they were rinsed in acid alcohol for 10 seconds, rinsed in 95% alcohol briefly, dehydrated in 95% alcohol for 5 minutes and 100% alcohol for 10 minutes and cleared using the clearing agent CitriSolv (Fisher Scientific) for 10 minutes. Slides were removed from the clearing agent and allowed to dry for several hours. Once dry, the slides were viewed under oil emersion at 100x magnification. *Thelohania solenopsae* spores were visible as pink, ovoid objects. In most situations, when present, spores were seen immediately and spread evenly throughout the slide. In the rare occasions in which spores were very sparse on the slide, I discarded the colonies and did not use them in my experiments. It took approximately five minutes per slide to accurately assess the presence or absence of *T. solenopsae*. Degree or percentage of infection was not determined in this study; only the presence or absence of *T. solenopsae* was deemed necessary for the study at hand. Infected colonies of RIFA were initiated in the same manner as healthy colonies as previously explained.

After colony set up, both RIFA and *M. minimum* were allowed to become accustomed to their new surroundings for a period of at least 24 hours. Invasion was initiated for healthy and infected RIFA colony sizes of 100, 300, 600, 800, and 1000 by inserting a glass tunnel into the shoe boxes in holes located 5, 15, and 25 cm from the edge of the shoe box and 1 cm from the bottom of the shoe box. The tubes were 10 cm in length, cut from the tips of nine inch disposable Pasteur Pipettes. The tips of the pipettes were not uniform in size on either end; therefore the holes drilled into the boxes were either 0.1 cm or 0.2 cm. The large or small holed boxes were randomly chosen for *M. minimum* or RIFA colonies. The glass tunnels were held in place by Plumbers

Goop™ (Eclectic Products, Pineville Louisiana), a contact adhesive and sealant that can be purchased at hardware stores. The adhesive also prevented accidental escape by either ant species. The Fluon® was wiped clean from the shoeboxes below each tunnel with a moist paper towel to ensure that both ant species would be able to climb to the tunnel. The pairing of RIFA and *M. minimum* colonies was randomly chosen without regard to colony or collection site.

Observations were recorded every hour for the first 12 hours and every 24 hours thereafter for a total of 31 days. After 31 days the colonies were allowed to continue to exist, but observations were taken once weekly. Colonies that still existed after 92 days were terminated. A single repetition of experimental colonies consisted of infected RIFA colony sizes of 100, 300, 600, 800, 1000 workers paired with one *M. minimum* colony of 1000 workers. A single control groups consisted of healthy RIFA colonies of the same worker numbers paired with *M. minimum* colonies of 1000 workers. The experiment was repeated five times producing a total of 50 *M. minimum* colonies, 25 healthy RIFA and 25 unhealthy RIFA colonies.

Events that were determined to be invasive events performed by *M. minimum* included: entering the tunnel (or tunneling), entrance into RIFA boxes, the presence of *M. minimum* workers within 2 cm of RIFA nests, the presence of *M. minimum* on RIFA nests, and the presence of *M. minimum* inside RIFA nests. Because the nests of *M. minimum* were open and covered with a construction paper tent, it was difficult to determine if RIFA performed certain invasive events against *M. minimum*. Therefore the same events deemed invasive events executed by *M. minimum* could not be applied

as invasive events performed by RIFA against *M. minimum*. Therefore, the only invasive event of RIFA that was recorded was the entrance of RIFA into *M. minimum* boxes. The death of either colony was also observed and recorded.

In order to determine if *M. minimum* was deterred from invading RIFA as their colony sizes increased in worker number, analysis of variance was performed, analyzing individually the time for each invasive event to occur by *M. minimum* for infected RIFA colonies and the time for each invasive event to occur in healthy colonies. In order to determine which colony sizes in particular significantly differed in the time it took *M. minimum* to perform invasive events against them, least significant difference (LSD) was used. Analysis of variance was also utilized in order to determine if colonies infected with *T. solenopsae* are more susceptible to invasion by *M. minimum*, comparing healthy and unhealthy RIFA colonies for all five invasive events listed previously. For the final objective, analysis of variance was once again used to determine the invasiveness of *M. minimum* and RIFA comparing the time in days for either species to enter the opposing ants box. All statistical analysis was performed using SPSS for Windows version 13.0 (SPSS Inc. 2003).

RESULTS

After 92 days of observation, six of 50 (12%) RIFA colonies died due to *M. minimum* invasion. Those colonies included sizes of 100, 800 and 1000 RIFA workers. Of the RIFA colonies that died, three were healthy (sizes of two 100 and one 800 worker colonies) and three were infected with *T. solenopsae* (sizes of 100, 800, and 1000 worker colonies). The number of *M. minimum* colonies out of 50 that died after 92 days was nine (18%). *Monomorium minimum* colonies that died were invaded by infected RIFA colonies of 600, two 800, and three 1000 worker colonies and those invaded by healthy RIFA included sizes of 800, and two 1000 worker colonies. Surviving RIFA and *M. minimum* colonies exhibited a tolerance for one another after a mean of 4.96 days in healthy RIFA colonies and 6 days in infected colonies and, except for the aforementioned cases, continued to coexist peacefully throughout the remainder of the study.

It was observed that *M. minimum* engaged in combat with RIFA workers by standing still and following them with their raised abdomens, attempting to sting RIFA workers. I observed that *M. minimum* has the ability to kill RIFA workers with their stinger through envenomization. *Monomorium minimum* commonly exhibited thanotosis when in the close presence of more than five RIFA workers by lying still, appearing to be dead. This behavior apparently served to protect them from RIFA attack and was noticed most frequently when *M. minimum* workers were not in the presence of fellow

nest mates, and when on or in the RIFA nest. *Monomorium minimum* would resume activity once RIFA workers were no longer a threat.

Once an invasion had been initiated by *M. minimum*, RIFA workers immediately withdrew to their nest. This action may have been to protect the queens and brood. When *M. minimum* workers had retreated back to their box, RIFA emerged to forage and defend their colony. When over 100 *M. minimum* workers were present in the RIFA box, RIFA seemed to be confined to their nest, did not emerge to forage and usually fewer than 10 emerged to defend. Most defensive actions by RIFA occurred within 2 cm of the RIFA nest, on the nest, or in the nest.

The mean times until an invasive event occurred by *M. minimum* against each healthy RIFA colony size is illustrated in Figure 2. Figure 2 is a bar chart showing that, in general, there was a relationship between the RIFA colony size and time until *M. minimum* executes an invasive event. In general, the larger the healthy RIFA colony size, the longer an invasive event took to occur. There was a significant difference in the time for *M. minimum* to get on to RIFA nests ($f = 0.022$) and enter RIFA nests ($f = 0.031$) of at least one RIFA colony size. There was no significant difference in the ability of *M. minimum* to tunnel ($f = 0.193$), enter the RIFA box ($f = 0.228$), and get within 2 cm of the RIFA nest ($f = 0.073$).

Figure 3 is a bar chart illustrating the mean time for *M. minimum* to perform an invasive event against different sizes of RIFA colonies infected with *T. solenopsae*. RIFA colonies of 100, 300 and 600 show a general trend where it takes *M. minimum* longer to perform invasive events against larger colonies. However, for *M. minimum*

colonies of 800 and 1000 there is actually a decrease in the amount of time it takes *M. minimum* to invade. The general trend found in healthy RIFA colonies if not evident in infected RIFA colonies. There was no significant difference in the ability of *M. minimum* to perform any invasive event against RIFA colonies infected with *T. solenopsae* (tunnel f = 0.656, in box f = 0.634, near nest f = 0.674, on nest f = 0.337, in nest f = 0.437) when comparing smaller and larger colonies.

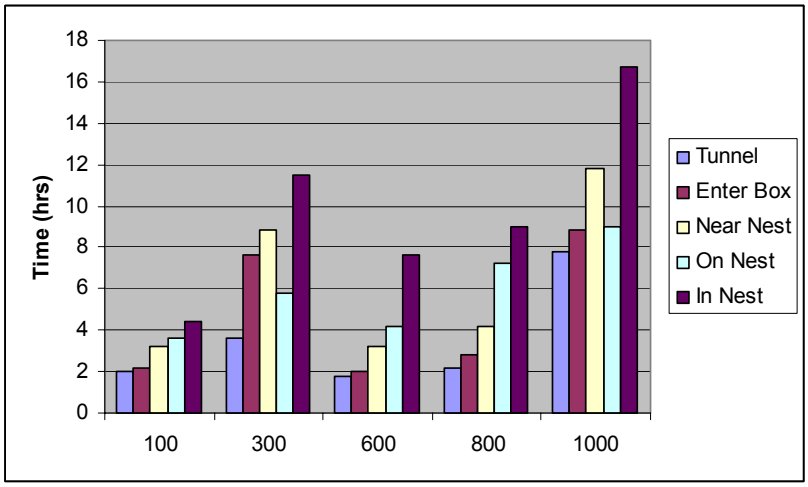

Figure 2. The mean time in hours for invasive events performed by *Monomorium minimum* on healthy *Solenopsis invicta* colonies of varying sizes to occur. Each bar represents an invasive event. *In some cases an invasive event never occurred, as a result only four of five observations were averaged. This explains why some earlier events have larger mean times than later events.

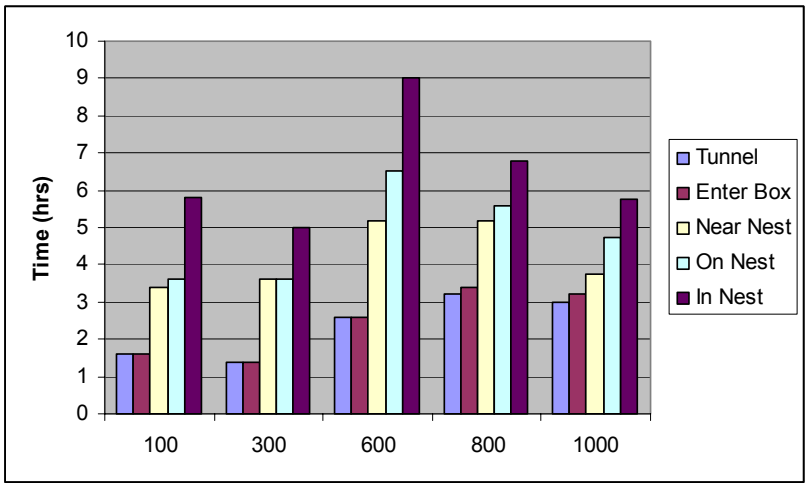

Figure 3. The mean time in hours for invasive events performed by *Monomorium minimum* on *Solenopsis invicta* colonies infected with *Thelohania solenopsae* of varying sizes to occur. Each bar represents an invasive event.

In order to determine exactly which RIFA colony sizes significantly differed from one another, Least Significant Difference (LSD) was utilized to compare the time *M. minimum* took to accomplish and invasive event for each RIFA colony size of healthy and infected colonies. The results are illustrated in Tables 1 and 2. The time for *M. minimum* to enter the tunnel placed between the two boxes differed significantly between RIFA colony sizes of 100 and 1000 and 600 and 1000. There was no significant difference in the time for *M. minimum* to enter RIFA boxes, between any RIFA colony sizes. The time for *M. minimum* to reach within 2 cm of the RIFA nest differed significantly between colony sizes of 100 and 1000, 600 and 1000, and 800 and 1000. The time for *M. minimum* to reach the RIFA nest significantly differed for RIFA colony sizes of 100 and 800, 100 and 1000, and 600 and 1000. The time for *M. minimum* to enter the RIFA nests differed significantly between RIFA colony sizes of 100 and 1000, 600 and 1000, and 800 and 1000.

Table 1. The mean time in hours for *Monomorium minimum* to perform invasive events against healthy *Solenopsis invicta* colonies of varying sizes.

RIFA Colony Size	Tunnel	Enter RIFA Box	Near RIFA Nest	On RIFA Nest	In RIFA Nest
100	2.00 ac	2.20 a	3.20 a	3.60 a	4.40 a
300	3.60 abc	7.60 a	8.80 abc	5.75 abc*	11.50 ab*
600	1.80 abc	2.00 a	3.20 ab	4.20 abc	7.60 a
800	2.20 ac	2.80 a	4.20 ab	7.00 bc	8.40 a
1000	3.40 b	4.40 a	8.75 c*	8.75 d*	14.33 b*

* Means only include four of five colonies in the experiment. In these situations the invasive event never occurred.
[1]Means followed by different letters in a column are significantly different at α = .05

Table 2. The mean time in hours for *Monomorium minimum* to perform invasive events against *Solenopsis invicta* colonies infected with *Thelohania solenopsae* of varying sizes.

RIFA Colony Size	Tunnel	Enter RIFA Box	Near RIFA Nest	On RIFA Nest	In RIFA Nest
100	1.60 a	1.60 a	3.40 a	3.60 a	5.80 a
300	1.40 a	1.40 a	3.60 a	3.60 a	5.00 a
600	2.60 a	2.60 a	5.20 a	6.50 a*	9.00 a*
800	3.20 a	3.40 a	5.20 a	5.60 a	6.80 a
1000	3.00 a	3.20 a	3.75 a*	4.75 a*	5.75 a*

* Means only include four of five colonies in the experiment. In these situations the invasive event never occurred.
[1]Means followed by different letters in a column are significantly different at α = .05

The mean times for *M. minimum* to perform an invasive event in healthy RIFA colonies and colonies infected with *T. solenopsae* are illustrated in Tables 1 and 2. There was a significant difference in the time it took *M. minimum* to get on the RIFA nest ($f = 0.047$) and enter the RIFA nest ($f = 0.049$) when comparing healthy and infected colonies of 1000 workers. In these situations the time for *M. minimum* to perform the invasive event took longer to occur in healthy colonies than unhealthy colonies, see Figure 4. *Monomorium minimum* also tunneled significantly faster into RIFA colonies of 300 workers infected with *T. solenopsae* than healthy colonies of 300

workers (f = 0.023). All other RIFA colony sizes and invasive event combinations showed no significant difference (see Appendix, Table 3)

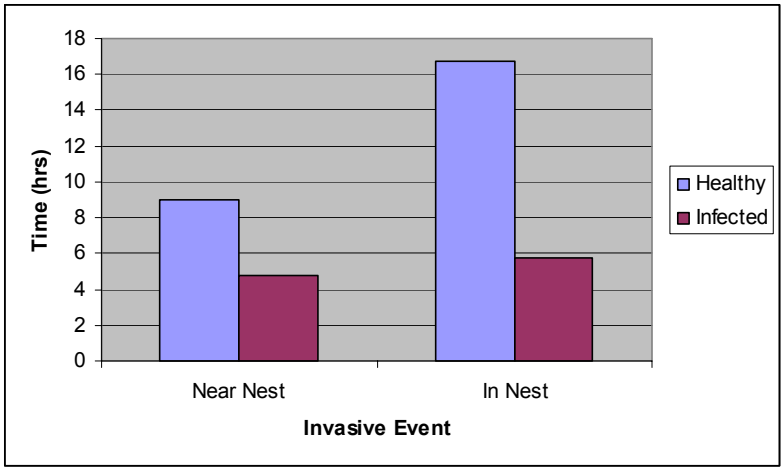

Figure 4. The mean time in hours for *Monomorium minimum* to perform an invasive event in *Solenopsis invicta* colonies infected with *Thelohania solenopsae* and healthy *Solenopsis invicta* colonies of 1000 workers.

In all experimental cases, *M. minimum* was the first ant species to enter the opposing ant's nest. In order to determine which ant species was the most invasive, the time until either ant species entered the opposing ants nest was compared between RIFA and *M. minimum*. *Monomorium minimum* entered RIFA's box at every RIFA colony size whether infected or healthy. The mean time for *M. minimum* to enter RIFA boxes was one day (Figure 5). The mean time for all RIFA colony sizes to enter the box of *M. minimum* was 22.67 days for healthy colonies, 17.24 days for colonies infected with *T. solenopsae*, and 19.74 for all RIFA colonies, regardless of their status of infection.

Monomorium minimum entered the opposing ant's box significantly sooner than all RIFA colonies combined (f = 0.001). *Monomorium minimum* is a significantly more

invasive ant species when compared to healthy RIFA colonies (f = 0.001) and when compared to infected RIFA colonies (f = 0.001). Figure 5 illustrates the comparison of the invasive abilities of RIFA and *M. minimum*, showing the difference in the amount of time for either ant to enter the opposing ant's nest.

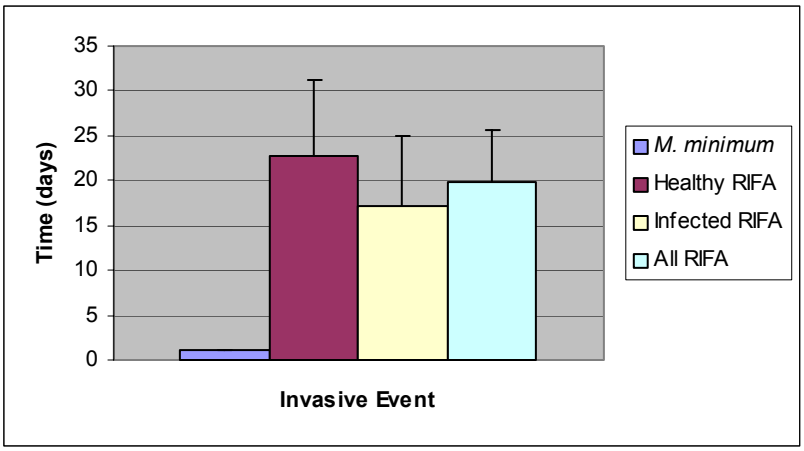

Figure 5. The mean time in days for *Monomorium minimum*, uninfected *Solenopsis invicta*, *Thelohania solenopsae* infected *Solenopsis invicta*, and all *Solenopsis invicta* colony combinations to initiate invasion by entering the opposing ant's box.

DISCUSSION AND CONCLUSIONS

The percentage of RIFA colonies that died during the course of this experiment was relatively low, 12% of healthy colonies and 12% of colonies infected with *T. solenopsae*. Therefore, it cannot be ascertained from this study that *M. minimum* alone or in conjunction with *T. solenopsae* would be effective in controlling RIFA colonies because so few RIFA colonies died. However, because *M. minimum* performed all invasive events toward every RIFA colony size, all colony sizes were susceptible to invasion by *M. minimum*.

The percentage of *M. minimum* colonies that died was also relatively low, 18%, suggesting that RIFA held in close proximity to *M. minimum* nests is not a consistent threat to the survival of *M. minimum* colonies. These findings, and the fact that the majority of the ant colonies could live with one another without major combat support previous reports that *M. minimum* and RIFA can and do coexist in the same areas (Stein and Thorvilson 1989, Porter and Savignano 1990, Helms and Vinson 2001).

By studying the invasive interactions between *M. minimum* and RIFA infected with *T. solenopsae*, it appears that *T. solenopsae* causes a change in the defensive behavior of RIFA. It can be assumed that the ability of *M. minimum* to invade RIFA colonies is dependent upon the ability of RIFA to defend their colony. It was shown that larger RIFA colonies had more workers available to defend the colony; therefore, it was harder for *M. minimum* colonies to perform such invasive events as to reach and enter

the RIFA nest. Colony size has also been shown to affect invasion time by native ants in a previous study using smaller RIFA colony sizes (Rao and Vinson 2004).

In this study I found that RIFA colony size significantly affected the ability of *M. minimum* to perform certain invasive events for healthy colonies, but did not significantly affect the ability of *M. minimum* to perform any invasive event for colonies infected with *T. solenopsae*. This suggests that the stress caused by *T. solenopsae* infection negatively affects the ability of RIFA to defend their nest and prevent *M. minimum* from entering. The results of Objective 1 caused the rejection the null hypothesis that the colony size of healthy RIFA does not have a significant effect on the time to perform invasive events by *M. minimum*. The null hypothesis, however, was accepted for RIFA colonies infected with *T. solenopsae*.

When comparing the effect of RIFA colony size on the ability of *M. minimum* to perform invasive events, only the time to reach the RIFA nest and enter the nest showed a significant difference between groups. This is most likely because *M. minimum* are extremely invasive ants, and once a path to a new resource is made available, they will almost immediately utilize it; therefore, events such as tunneling and entering the box have no association with RIFA or their defensive behavior. Invasive events, such as nearing, reaching, and entering the RIFA nests are more likely to be true invasive events because confrontation with multiple RIFA workers is inevitable.

Using LSD to make pairwise comparisons among the RIFA colony sizes indicated the pairs that were significantly different always include colonies of 1000 RIFA workers in the times for *M. minimum* to near the nest, reach the nest, and enter the

nest. Therefore, healthy RIFA colonies of 1000 workers are able to prevent certain invasive events to occur (near the nest, reach the nest and enter the nest) whereas, healthy colonies of 100, 600 and 800 are generally unable to prevent these invasive events from occurring.

Healthy RIFA colonies of 300 workers did not differ significantly in the time until an invasive event occurred when compared to healthy RIFA colonies of 1000 workers. This can be explained when looking at the individual replications. During the fourth replication, the time for *M. minimum* to enter the box or get near the nest did not occur until 24 hours had passed, skewing the data. Invasive events such as nearing and entering the RIFA nest never occurred, which also skewed the data and reduced the sample size used for statistical analysis from five *M. minimum* populations to four. This population caused the mean to rise and increased the variance. This also explains why there was significant difference in the time for *M. minimum* to tunnel when comparing healthy and infected RIFA colonies.

In general, the mean times for an invasive event to take place took less time to occur for infected RIFA colonies than for healthy colonies. However, only colonies of 1000 RIFA workers showed a significant difference in the time for *M. minimum* to reach and enter the RIFA nest. This may be because colonies of less than 1000 workers had too few workers available to defend the colony, and therefore, whether or not the colony was debilitated from microsporidian infection did not make a difference. Colonies of 1000 workers were larger in size and may have been better able to protect different parts of their territory. However, RIFA colonies infected with *T. solenopsae* of 1000 workers

are not capable of protecting their territory and *M. minimum* is able to reach and enter the nest more quickly than they can in healthy RIFA colonies. This is also supported by the LSD results for healthy colonies when comparing the time for an invasive event to occur between colony sizes; healthy RIFA colonies of 1000 are better able to prevent *M. minimum* to from nearing, reaching and entering their nest when compared to smaller colony sizes.

Based on the results of Objective 2, I rejected the null hypothesis that RIFA colonies infected with *T. solenopsae* are more susceptible to invasion by *M. minimum*. This is only true for RIFA colonies of 1000 workers, however, and the null hypothesis is supported for RIFA colonies of 100, 300, 600, and 800 workers. As with Objective 1, the results of Objective 2 also suggests that *T. solenopsae* alters the defensive abilities of RIFA because large infected colonies are unable to prevent competing ants from entering their territory as quickly when compared to large healthy RIFA colonies.

This study demonstrated conclusively that *M. minimum* is an invasive ant species. The initiation of invasion was determined to be the entrance into the opposing ant's nest because it was the first time contact was made with the opposing ant and was the first step to nearing, reaching, or entering the opposing ant's nest. In addition, the nests were very different; therefore, nearing or entering the nest would not have been a fair estimation. In every case, *M. minimum* entered RIFA's box, regardless of colony size or status of infection. RIFA, however, did not enter *M. minimum*'s box in every case (72% of healthy and 84% of infected colonies). Regardless of whether RIFA was healthy or infected with parasites, RIFA was significantly the less invasive ant with

regards to the time to initiate invasion. This is a promising result, because RIFA is commonly believed and reported to be an extremely invasive ant, pushing other native ants out the area in which they inhabit and this proves that there are native ants that can successfully compete with RIFA (Porter and Savignano 1990).

This study holds promise in biological control of the red imported fire ant. It has already been discovered that the effects of *T. solenopsae* alter RIFA in physiological and biological ways (Williams et al. 1999, Cook 2002, Overton 2003). These changes will undoubtedly put stress on an organism, changing its behavior. This study suggests that *T. solenopsae* alters the defensive behavior of RIFA by inhibiting infected colonies from defending their nest as well as healthy colonies.

Ants infected with microbial pathogens exhibit such behaviors as grooming, nest hygiene, and avoidance (Oi and Periera 1992). This may explain why RIFA did not exit their nest or defend their nest as well when infected with *T. solenopsae* compared to healthy colonies. Because *T. solenopsae* is an internal parasite, it seems unlikely that grooming would be a behavior caused by infection. However, it is likely that infected individuals may avoid contact with *M. minimum* in an attempt to protect themselves from attack, explaining the results of the first two objectives.

Monomorium minimum have been shown to be predators of founder queens, therefore, conservation of these native ants may be beneficial to the control of RIFA (Nichols and Sites 1991). As *T. solenopsae* becomes more widespread, infected queens trying to found new colonies may find it difficult to ward off other organisms competing for the same food and territory. If *M. minimum* is conserved in areas in which they

already inhabit or introduced into areas they do not, it may be likely that RIFA will be unable to successfully colonize that area. *Monomorium minimum* may better act as prevention for RIFA infestation than as a true biological control agent. Furthermore, conservation of *M. minimum* would be beneficial because it has been suggested that the best source of control for RIFA is to preserve native ant species that are competitors, and this study demonstrated that *M. minimum* is a successful competitor of RIFA (Drees et al. 1996).

More in depth studies need to be performed with regards to this experiment in order to truly establish the impacts of *M. minimum* and *T. solenopsae* on RIFA colonies. RIFA colony sizes of greater than 1000 workers should be utilized in order to determine if the presence of *T. solenopsae* impacts the ability of *M. minimum* to invade larger RIFA colonies. Another modification to this study would be to use *M. minimum* colonies of different sizes to establish if larger colonies are more likely to cause more damage to RIFA colonies and if smaller colonies are less likely to invade. This study only used *M. minimum* colonies from two different locations, due to the availability of the ant and because they are so cryptic is was difficult to locate their nests. Using colonies from different sites may produce more diverse results because different colonies could exhibit dissimilar behaviors, defensively and otherwise.

Colony relocation to other areas is a common response of RIFA when encountering parasites and pathogens (Oi and Periera 1992). In this experiment neither RIFA nor *M. minimum* were given the opportunity to move from their original boxes. If either ant were given another avenue to another site away from the opposing ant species,

it may have been discovered that one or both ant species may move in order to avoid confrontation. This would support the claims that *M. minimum* and RIFA are able to coexist and nest near one another without either ant causing noticeable harm to the other (Porter and Savignano 1990, Helms and Vinson 2001). If this study was to be repeated or altered, providing the ants access to alternate boxes may more accurately reflect their behaviors in the wild.

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APPENDIX A

STATISTICS FROM OBJECTIVE 1 DATA: ABILITY OF *Monomorium minimum* TO INVADE VARYING SIZES OF HEALTHY *Solenopsis invicta* COLONIES AND *Solenopsis invicta* COLONIES INFECTED WITH *Thelohania solenopsae*

Table A1. Analysis of variance results comparing the ability of *Monomorium minimum* to perform invasive events against *Solenopsis invicta* colonies of varying sizes.

Invasive Event	Healthy RIFA Colonies	RIFA Colonies Infected with Thelohania solenopsae
Tunnel	0.193	0.656
Enter RIFA Box	0.228	0.634
Near RIFA Nest	0.073	0.674
On RIFA Nest	0.022	0.337
In RIFA Nest	0.031	.0437

Table A2. Fisher's Least Significant Difference results from healthy *Solenopsis invicta* colonies. Significance indicates a difference in the time for *Monomorium minimum* to perform an invasive event against varying *Solenopsis invicta* colony sizes.

Dependent Variable	Size (I)	Size (J)	Mean Difference	Standard Error	Significance
Tunneling	100	300	-1.600	2.740	0.566
		600	0.200	2.740	0.943
		800	-0.200	2.740	0.943
		1000	-5.800*	2.740	0.047
	300	100	1.600	2.740	0.566
		600	1.800	2.740	0.519
		800	1.400	2.740	0.615
		1000	-4.200	2.740	0.141
	600	100	-0.200	2.740	0.943
		300	-1.800	2.740	0.519
		800	-0.400	2.740	0.885
		1000	-6.000*	2.740	0.041
	800	100	0.200	2.740	0.943
		300	-1.400	2.740	0.615
		600	0.400	2.740	0.885
		1000	-5.600	2.740	0.054

	1000	100 300 600 800	5.800* 4.200 6.000* 5.600	2.740 2.740 2.740 2.740	0.047 0.141 0.041 0.054
Enter RIFA Box	100	300 600 800 1000	-5.400 0.200 -0.600 -6.600	3.703 3.703 3.703 3.703	0.160 0.957 0.873 0.090
	300	100 600 800 1000	5.400 5.600 4.800 -1.200	3.703 3.703 3.703 3.703	0.160 0.146 0.210 0.749
	600	100 300 800 1000	-0.200 -5.600 -0.800 -6.800	3.703 3.703 3.703 3.703	0.957 0.146 0.831 0.081
	800	100 300 600 1000	0.600 -4.800 0.800 -6.00	3.703 3.703 3.703 3.703	0.873 0.210 0.831 0.121
	1000	100 300 600 800	6.600 1.200 6.800 6.000	3.703 3.703 3.703 3.703	0.090 0.749 0.081 0.121
Near RIFA Nest	100	300 600 800 1000	-5.600 0.000 -1.000 -8.600*	3.452 3.452 3.452 3.452	0.120 1.000 0.775 0.022
	300	100 600 800 1000	5.600 5.600 4.600 -3.000	3.452 3.452 3.452 3.452	0.120 0.120 0.198 0.395
	600	100 300 800 1000	0.000 -5.600 -1.000 -8.600*	3.452 3.452 3.452 3.452	1.000 0.120 0.775 0.022
	800	100 300 600 1000	1.000 -4.600 1.000 7.600*	3.452 3.452 3.452 3.452	0.775 0.198 0.775 0.040
	1000	100 300 600 800	8.600* 3.000 8.600* 7.600*	3.452 3.452 3.452 3.452	0.022 0.395 0.022 0.040
On RIFA Nest	100	300 600 800 1000	-2.150 -0.600 -3.600* -5.400*	1.624 1.531 1.531 1.624	.0.202 0.700 0.030 0.004
	300	100 600	2.150 1.550	1.624 1.624	0.202 0.353

		800	-1.450	1.624	0.384
		1000	-3.250	1.712	0.074
	600	100	0.600	1.531	0.700
		300	1.550	1.624	0.353
		800	-3.00	1.531	0.066
		1000	-4.800*	1.624	0.008
	800	100	3.600*	1.531	0.030
		300	1.450	1.624	0.384
		600	3.000	1.531	0.066
		1000	-1.800	1.624	0.282
	1000	100	5.400*	1.531	0.030
		300	3.250	1.624	0.384
		600	4.800*	1.531	0.066
		800	1.800	1.624	0.282
In RIFA Nest	100	300	-7.100	3.529	0.059
		600	-3.200	3.327	0.349
		800	-4.600	3.327	0.184
		1000	-12.350*	3.529	0.003
	300	100	7.100	3.529	0.059
		600	3.900	3.529	0.284
		800	2.500	3.529	0.488
		1000	-5.250	3.720	0.175
	600	100	3.200	3.327	0.349
		300	-3.900	3.529	0.284
		800	-1.400	3.327	0.679
		1000	-9.150*	3.529	0.018
	800	100	4.600	3.327	0.184
		300	-2.500	3.529	0.488
		600	1.400	3.327	0.679
		1000	-7.750	3.529	0.041
	1000	100	12.350*	3.529	0.003
		300	5.250	3.720	0.175
		600	9.150*	3.529	0.018
		800	7.750*	3.529	0.041

* Means followed by an asterisk are statistically significant at $\alpha = 0.05$.

APPENDIX B

STATISTICS FROM OBJECTIVE 2 DATA: COMPARISON OF *Solenopsis invicta* COLONIES INFECTED WITH *Thelohania solenopsae* AND HEALHTY *Solenopsis invicta* COLONIES

Table B1. Analysis of variance results comparing the ability of *Monomorium minimum* to perform invasive events against healthy *Solenopsis invicta* colonies and *Solenopsis invicta* colonies infected with *Thelohania solenopsae* of varying sizes.

RIFA Colony Size	Tunnel	Enter RIFA Box	Near RIFA Nest	On RIFA Nest	In RIFA Nest
100	0.659	0.545	0.874	1.000	0.427
300	0.023	0.176	0.224	0.176	0.147
600	0.545	0.644	0.308	0.286	0.559
800	0.497	0.690	0.634	0.359	0.243
1000	0.306	0.231	0.075	0.047	0.049

VITA

Molly Elizabeth Keck was born in San Antonio, TX on July 12, 1981. She is the daughter of Russell and Martha Bush and has three younger brothers, Neal, Matt, and Ben. Molly married Casey Lee Keck in the summer of 2004.

Molly graduated from Texas A&M University with a double major in Biology and Entomology in May of 2003 and began to pursue her Masters degree in Entomology in the summer of 2003 under Dr. Roger E. Gold. During her time in graduate school, Molly was a teaching assistant for Dr. Gold's course, Insects in Human Society, for five semesters.

Upon receiving her Masters degree in May of 2005, Molly accepted a position as an Extension Specialist for the Texas Cooperative Extension Service in San Antonio, TX. Her permanent address is:

6043 Woodway Place

San Antonio, TX 77849